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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SCB828PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/14719	International filing date *(day/month/year)* 22.12.2003	Priority date *(day/month/year)* 23.12.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant ISTITUTO NAZIONALE PER LO STUDIO E LA CURA.. et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.07.2004	01.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Knudsen, H Telephone No. +49 89 2399-8696

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/14719

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-13	as originally filed

Claims, Numbers

6 (part), 7-11	as originally filed
1-5, 6 (part)	filed with telefax on 20.01.2005

Drawings, Sheets

1/3-3/3	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document/s/:

D1: EP-A-1 158 055 (ANKER PHILIPPE ; STROUN MAURICE (CH); CHEN XU QI (US)) 28 November 2001 (2001-11-28)

D2: WO 99/41406 A (UNIV MARYLAND) 19 August 1999 (1999-08-19)

D3: KOK DE J B ET AL: "Real-time quantification of human telomerase reverse transcriptase mRNA in tumors and healthy tissues" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 46, no. 3, March 2000 (2000-03), pages 313-318, XP002176598 ISSN: 0009-9147

NOVELTY:

D1 discloses an assay for determining the amount of RNA encoding hTERT enzyme in plasma or serum (see claim 1 and [0017]. The assay may be carried out as RT-PCR [0016] and the result analysed by electrophoresis [0020].

D2 discloses assays for determining the amount of RNA encoding hTERT enzyme or hTERT enzyme activity in plasma or serum (see claim 1, page 12 and p.13, last paragraph and Example 2. The assay may be carried out as RT-PCR and the result analysed by electrophoresis.

Thus, the method of claim 1 differs from both D1 and D2 in that it determines total circulating DNA in a plasma sample instead of RNA and employs a molecular beacon labelled with fluorophore and quencher and measures the fluorescence for the detection of the amplified DNA. Claims 1-11 are therefore considered novel.

INVENTIVE STEP:

In view of the results shown in the application and the scientific article "Journal of Clinical Oncology, vol. 21(21), p.3891-3893, (2003), it appears that the method of claim 1 has a sensitivity and specificity higher than the prior art methods for determining cancer via plasma DNA.

The prior art documents, D1 and D2, explain that DNA encoding certain proteins when found in blood samples are known markers of tumours and that the overexpression of hTERT (see D3) differs from the DNA markers in that it is a marker of all tumour types. However, none of the prior art documents suggest that the determination of genomic DNA of hTERT in blood samples represents a better tumour marker than the tumour markers of the prior art. Thus, the claimed solution to the problem of providing a better tumour marker would not be obvious to the skilled person and claims 1-11 therefore are considered inventive.

INDUSTRIAL APPLICABILITY:

Present claims 1-11 are considered industrially applicable.

FURTHER REMARKS:

It seems that the use of primers capable of amplifying both hTERT mRNA and DNA is not excluded from the claims. However, such primers would not be useful in determining the concentration of circulating total DNA, since the presence of RNA in the DNA preparation cannot be excluded. Claims 1-4 and 7-11 therefore lack clarity.

Enclosure 3

CLAIMS

1. A method for determining the concentration of circulating total DNA in a plasma sample from a cancer patient, a subject with cancer susceptibility or at risk of developing cancer, which comprises:

   1) extracting the DNA from the plasma sample;

   2) adding to the DNA preparation: a) a mixture of oligonucleotide primers suitable for PCR amplification of a fragment of the human telomerase reverse transcriptase (hTERT) gene, and b) an oligonucleotide probe, having at least one quencher and one reporter fluorophore at the 3' and 5' ends, able to anneal to a sequence within the region delimited by the primers,
   in suitable conditions for carrying out a PCR reaction,

   3) adding a heat-stable DNA polymerase with 5'-3' hexonuclease activity and amplifying the hTERT gene fragment;

   4) measuring the produced fluorescence.

2. A method according to claim 1, wherein the DNA concentration in the test sample is determined by interpolation of a calibration curve calculated with known amounts of DNA.

3. A method as claimed in claims 1-2, which further comprises comparing the concentration of circulating DNA to a reference concentration.

4. A method according to claim 3, wherein the reference concentration is from 9 to 25 ng/ml.

5. A method as claimed in claim 1, wherein said fragment of the human telomerase reverse transcriptase (hTERT) gene is from nt 13059 to nt 13156 of the sequence GenBank accession n. AF128893.

6. A method according to claim 5, wherein said fragment of the human telomerase reverse transcriptase (hTERT) gene is amplified using SEQ ID N.